

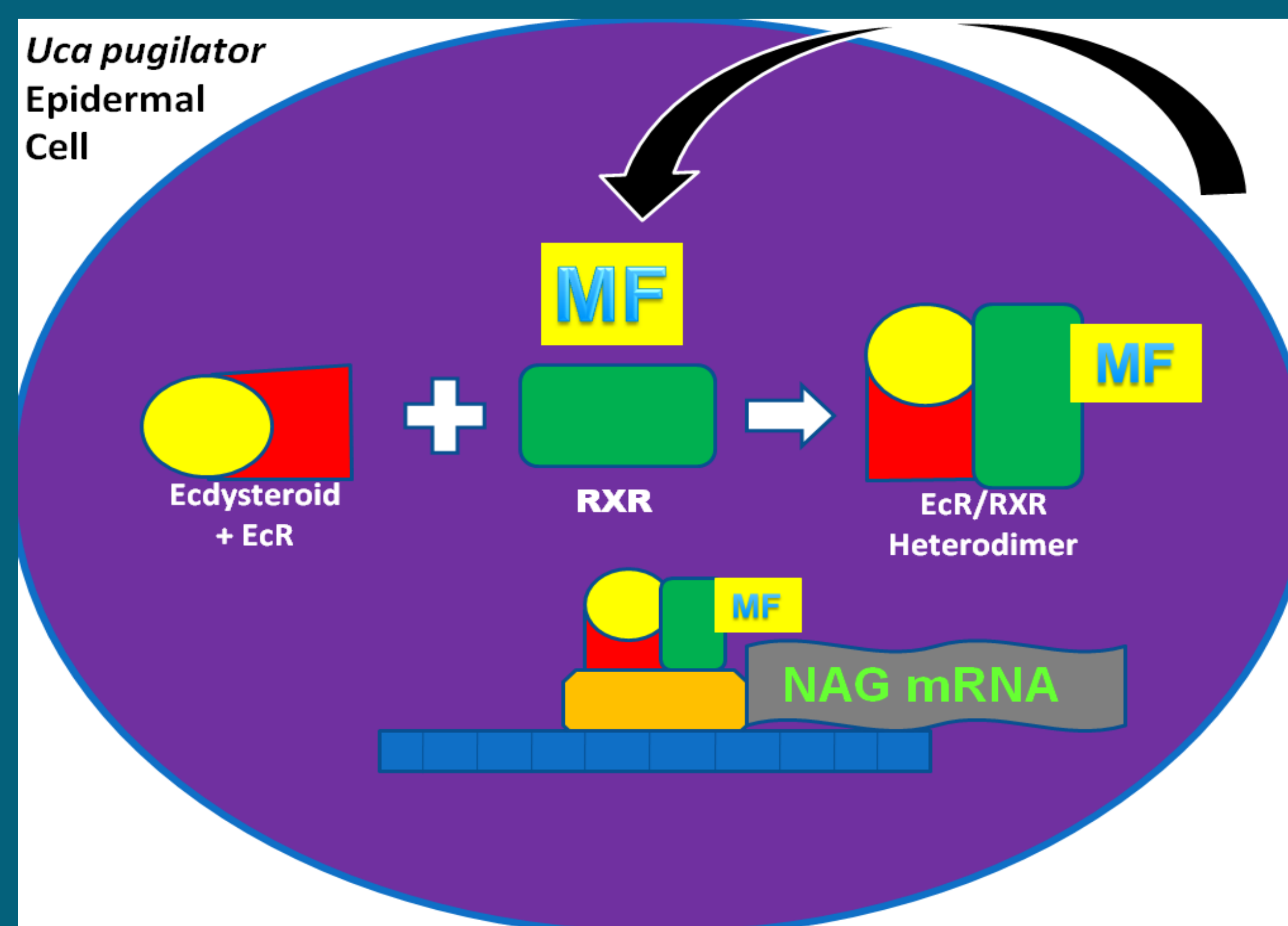
The exogenous methyl farnesoate does not impact epidermal ecdysteroid signaling *in vivo* in the fiddler crab, *Uca pugilator*

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Introduction

- Methyl farnesoate (MF) is a crustacean terpenoid hormone (Nagaraju 2007).
- However the receptor for MF remains equivocal.
- Retinoid X receptor has been proposed as the candidate receptor.
- Should MF signal through the RXR, the ecdysteroid signaling in the epidermis of the fiddler crab would be impacted after administration of the exogenous MF.
- Our emphasis was placed on two lines of *in vivo* evidence, the transcription of NAG gene and the activity of NAG.



Methods

Quantitation of Epidermal NAG mRNA

- Crabs were injected with 0, 0.2, 1, 5, or 20 ng/g wet weight MF
- RNA was extracted 1.5 hrs after injection
- Using real-time PCR, relative NAG mRNA quantity was calculated with the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen 2001)

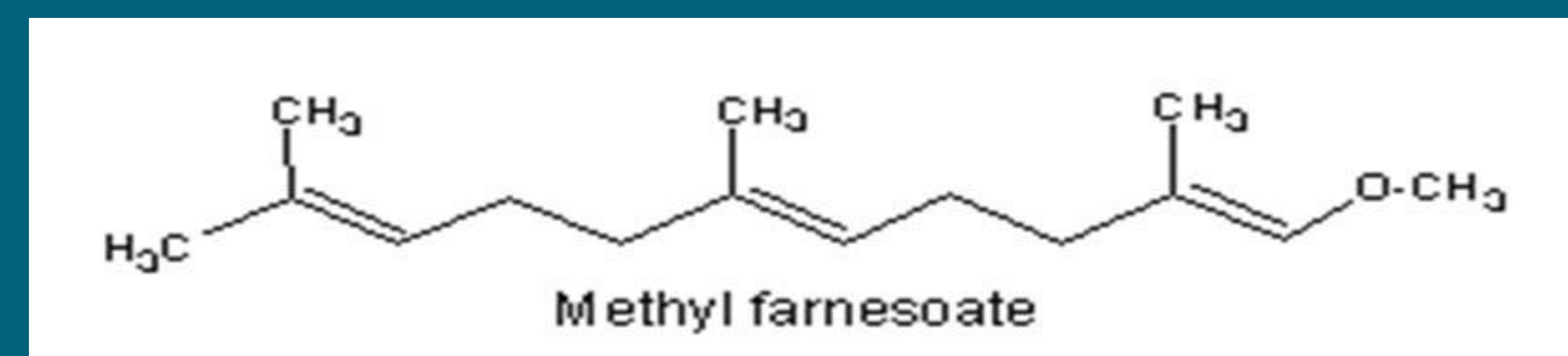
Assay of Epidermal NAG Activity

- Crabs were injected with 0, 20, or 2000 ng/g wet weight of MF on day 0
- On day 2 crabs were injected again with the same dosage of MF
- On day 4 crabs were sacrificed and epidermal tissue extracted
- A specific substrate for NAG, 4-nitrophenyl *N*-acetyl- β -D-glucosaminide (Sigma, St. Louis, MO), was added to homogenized epidermis
- Enzyme activity was expressed as μmol nitrophenol liberated ($\mu\text{g protein}^{-1}(20 \text{ min})^{-1}$) (Zou 2009)



Abstract

Methyl farnesoate (MF) is a crustacean hormone involved in various physiological processes. Because MF can bind to and alter the expression of crustacean retinoid X receptor (RXR), RXR has been proposed as a candidate receptor. This study investigated whether the exogenous MF impacts ecdysteroid signaling *in vivo* using N-acetyl- β -glucosaminidase (NAG) mRNA from epidermal tissue as a biomarker for ecdysteroid signaling. The NAG mRNA from fiddler crabs (*Uca pugilator*) injected with 0, 0.2, 1, 5, and 20 ng/g wet weight of MF was quantified, and an assay of epidermal NAG activity in crabs injected with 0, 20, and 2000 ng/g wet weight of MF was performed. Exogenous MF was found to have no effects on epidermal NAG gene transcription or NAG activity. These results show that MF is not capable of affecting epidermal ecdysteroid signaling, and are not supportive of the notion that MF signals through the RXR in Crustacea.



Results

- No statistically significant difference was observed in NAG mRNA from the epidermis of control crabs and those injected with 0.2, 1, 5, or 20 ng/g MF ($p = 0.948$, Fig. 1).
- No significant difference was seen between NAG activities in the epidermis of control crabs and those receiving two injections of 20 or 2000 ng/g MF ($p = 0.430$, Fig. 2).

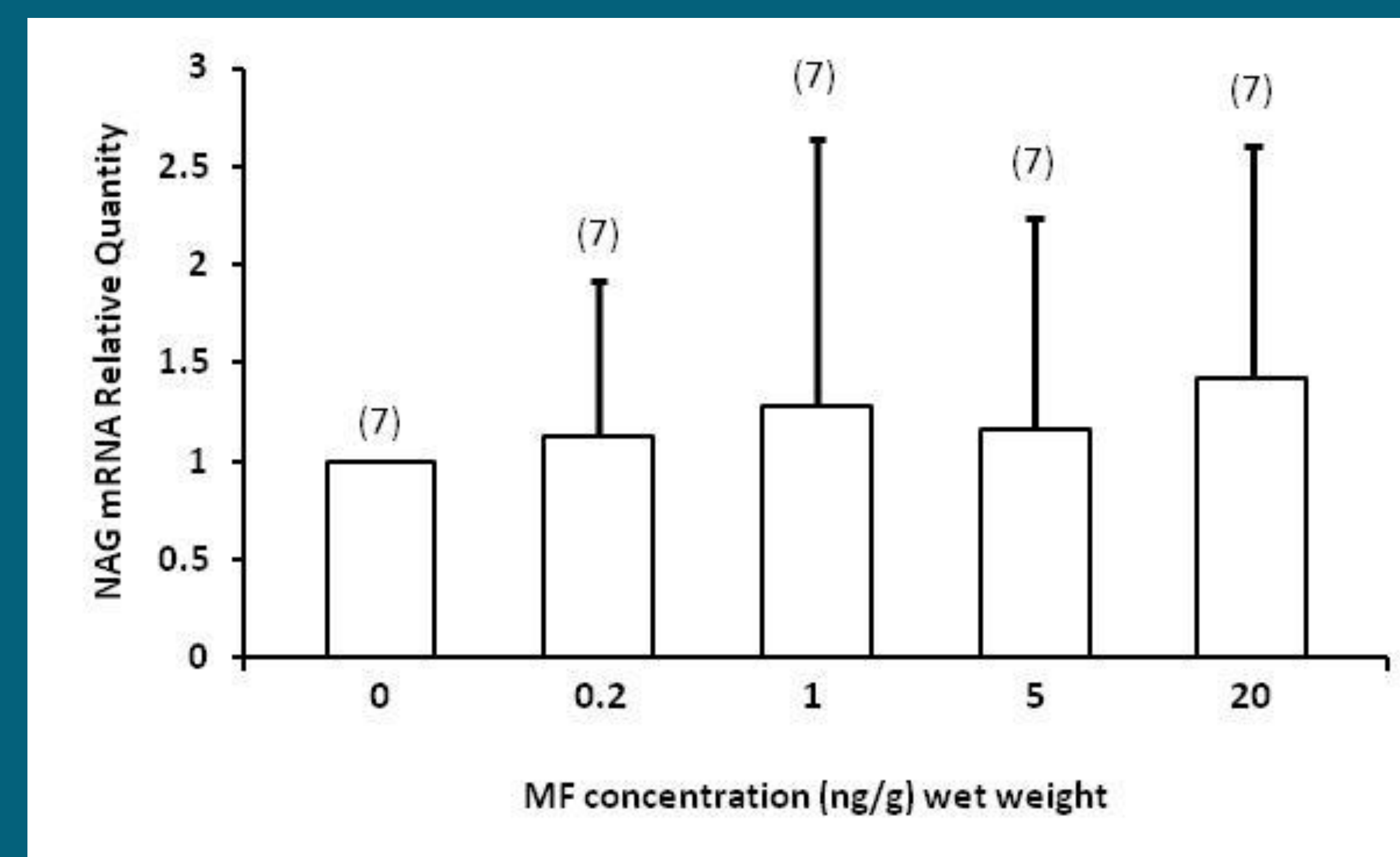


Figure 1: Relative quantity of epidermal NAG mRNA.

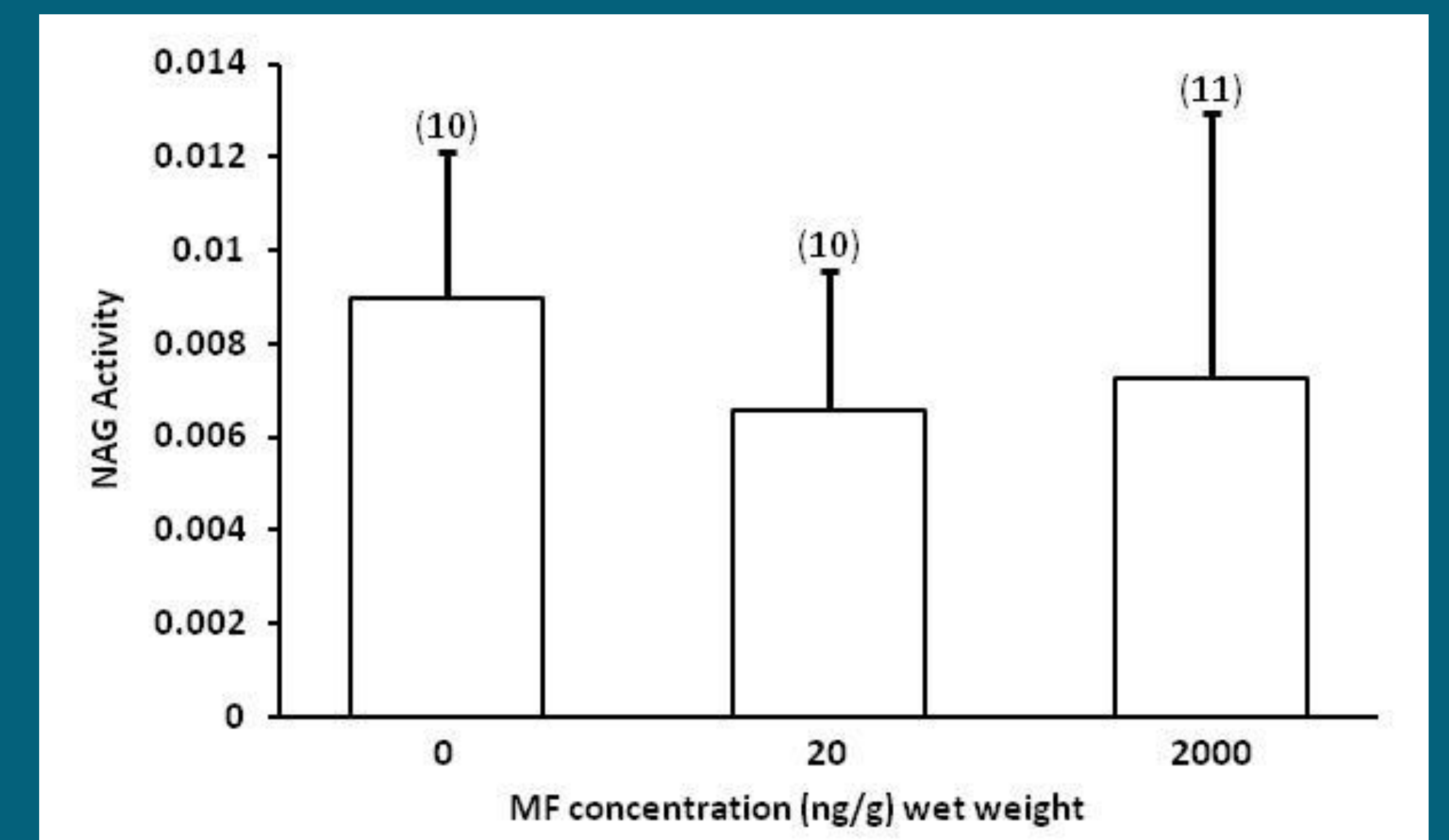


Figure 2. Activity of epidermal NAG. Activity expressed as μmol nitrophenol liberated ($\mu\text{g protein}^{-1}(20 \text{ min})^{-1}$).



Conclusions

Our results show that MF does not impact epidermal ecdysteroid signaling and that crustacean RXR is not the receptor for MF.

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